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Influence of Soil onents on the Transport of yclic Aromatic Hydrocarbon-Deg Bacteria through Saturate ous Media

MOHAMMED LAHLO
HAUKE HARMS,‡,#
DIRK SPRINGAEL,§
JOSÉ-JULIO ORTEG VO*,†

Instituto de Recursos Natura biología (IRNA), C.S.I.C., Apartado 1052, E-41080 Sevi Swiss Federal Institute for Environmental Science a logy (EAWAG), Überlandstrasse 133, CH-860 orf, Switzerland, and Vlaamse Instelling voor Tech Onderzoek (VITO), Boeretang 200, B-2400 Mol,

The transport of a selected acteria able to degrade polycyclic aromati arbons (PAHs) was investigated in saturated c periments, using as packing materials either a c ning soil or the isolated soil constituents montmoril y, sand, or soil humic acid-clay complexes. The bacterial strains studied were able to grow on phenanthrene, anthracene, fluorene, fluoranthrene, and pyrene and were characterized for cell size, aspect ratio, hydrophobicity, and zeta potential. Removal of bacteria from the transport solution was quantified by calculating relative adhesion efficiencies (α_t) for all combinations of bacteria and packing materials. Transport through soil varied from strain to strain. However, no clear relationship was observed between the studied physicochemical characteristics of the bacteria and their transport. The relative differences between strains observed in soil were conserved in column experiments with isolated clay. Coating the clay surfaces with two different soil humic acid fractions drastically increased the transport of all bacteria tested. The nonionic surfactant Triton X-100 facilitated transport of hydrophobic cells at concentrations above its critical micelle concentration but had no effect on the transport of hydrophilic bacteria. We suggest that clay is the main retarding agent of PAH-degrading bacteria in soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are priority environmental pollutants due to their frequent occurrence in soil and their significant toxicity and carcinogenicity. Catabolism of PAHs containing two to five aromatic rings has been reported to occur in the environment, and microbial degradation is seen as the most important pathway of their removal (*1*). In the subsurface, for example in soils and aquifers, most microorganisms occur in the attached state (*2*−*5*). This is due to the abundance of surfaces present and the strong tendency of microorganisms to adhere to and to grow in contact with surfaces. In soil, for a typical population of 10^7 to 10^8 cells per gram, and assuming that these organisms occur in microcolonies, the typical average distance between individual microcolonies is 100 μm (*6*). For microcolonies possessing the ability to degrade a certain contaminant, the average distances between them, and, as a consequence, their distances from a source of substrate, can be assumed to be even greater. Hence, consumption of nutrients and C-source in the neighborhood of the colonies has to be balanced by substrate resupply from relatively distant sources. This applies particularly to hydrophobic PAHs, the strong sorption of which reduces their bioavailability and limits their transfer. Bioremediation technologies are therefore often directed toward mobilizing nutrients and microorganisms in PAH-contaminated sites to overcome this limitation (*7*).

Despite their significant importance in bioremediation, the factors controlling the mobility of pollutant-degrading microorganisms are not well-known. Most of the laboratory studies on microbial transport in soil and aquifer materials have not focused on microorganisms selected for their catabolic ability but on bacteria belonging to common soil genera (*8*−*11*), on enteric bacteria (*12*), or on aquifer microorganisms (*13, 14*). Transport studies employing bacteria able to destroy specific organic pollutants are scarce (*15, 16*). The ability to degrade PAHs does not seem to be restricted to certain bacterial genera (*17, 18*). It is conceivable, however, that bacteria may themselves render hydrophobic contaminants more bioavailable when they possess specific cell surface components for substrate solubilization or whole-cell physicochemical properties that facilitate adhesion to the source of the chemical. The preference of some soil bacteria for hydrophobic surfaces may explain why different PAH-degrading bacteria were isolated when hydrophobic membranes, instead of a conventional water enrichment method, were used to enrich and recover the bacteria from soil (*19*).

Because bacteria have a low mobility in soil, when applied to soil surfaces they often penetrate only a few centimeters (*20*−*23*). However, several lines of evidence show that bacterial transport through soil can be increased by a variety of biological, chemical, and physical factors. Biological factors include properties of the bacterial populations that minimize their retardation by soil surfaces, such as reduced cell size (*15*), spherical shape (*14*), and hydrophilic cell surfaces (*24*). Higher organisms such as plants and earthworms may act as transporting agents (*21, 25*). Transport can also be facilitated by changes in the chemical composition of the carrier solution, such as higher pH (*13*), lower ionic strength (*9, 26, 27*), and the presence of surfactants (*28*) or natural organic matter (*29*). Finally, bacterial transport may be increased by soil textural properties which permit preferential water flow through macropores, such as the presence of large sand grains (*27*) and soil aggregates (*21*).

A factors often assumed to affect bacterial transport through soil is the presence of clay colloids. Amendment of sand (*11*) and soil (*30*) with clay resulted in a sharp reduction of bacterial transport through packed columns, as compared with the unamended material. This has been explained by the association between clay particles and bacteria that is frequently observed in suspension (*31*). However, it is difficult to attribute the influence of clay on bacterial transport solely

* Corresponding author phone: (+34) 95-4624711; fax: (+34) 5-4624002; e-mail: jjortega@irnase.csic.es.
† IRNA.
‡ EAWAG.
§ VITO.
# Present address: Swiss Federal Institute for Technology Lausanne (EPFL), IATE-Pédologie, GR- Ecublens, CH-1015 Lausanne, Switzerland.

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TABLE 1. Effect of Characteristics of PAH-Degrading Bacteria on Transport through Saturated Soil Columns

bacterium	substrate	mode of isolation[a]	cell width (μm)	cell length (μm)	aspect ratio[b]	θ_w[c,f] (deg)	ζ[d,f] (mV)	transport[e,f] C/C$_0$	α_t[g]	PV[h]
Sphingomonas sp. LH162	phenanthrene	L	0.4	1.2	3	77.0	−23.6	0.72 (0.99)	0.152 (0.003)	6.6 (25.1)
Mycobacterium sp. VM505	anthracene	L	0.8	1.2	1.5	35.3	−33.6	0.416 (0.908)	0.484 (0.053)	2.8 (23.6)
Mycobacterium sp. LB 307T	phenanthrene	T	0.8	1.0	1.25	85.7	−22.3	0.45 (0.57)	0.473 (0.326)	3.9 (24.8)
Arthrobacter polychromogenes	phenanthrene	L	0.4	1.6	4	28.2	−22.6	0.19 (0.35)	0.811 (0.515)	6.3 (24.8)
Mycobacterium sp. VM502	pyrene	L	1.2	1.6	1.33	86.3	−27.1	0.171 (0.245)	1.298 (1.033)	2.8 (23.6)
Sphingomonas sp. LB126	fluorene	L	1.0	1.2	1.2	23.4	−2.6	0.18 (0.18)	1.101 (1.140)	4.0 (24.8)
Mycobacterium sp. LB501T	anthracene	T	0.6	2.0	3.33	75.9	−60.2	0.11 (0.11)	1.33 (1.35)	3.0 (23.6)
Mycobacterium sp. LB208	pyrene	L	1.0	1.2	1.2	83.0	−40.6	0.04 (0.09)	2.079 (1.532)	1.7 (24.8)
Mycobacterium sp. VM503	fluoranthrene	L	0.8	1.6	2	80.0	−45.8	0.071 (0.072)	1.717 (1.704)	2.8 (23.6)

[a] L, liquid extraction; T, Teflon extraction. [b] Cell length divided by cell width. [c] θ_w, Contact angle. [d] ζ, zeta potential. [e] Final experimental values are given in parentheses as an indication of the dynamics of filter blocking. [f] Determinations were performed at pH 7.2. [g] α_t, adhesion efficiency. [h] PV, pore volume.

to adhesion, because the presence of clay within the soil matrix also affects the pore-size distribution and may give rise to direct interception of bacteria. Other than theoretical considerations and extrapolation of experimental results with liquid suspensions, little is known about clay-bacteria interactions in porous media (*32*).

Recently, we reported on a new method for conducting bacterial transport experiments in clay-rich, aggregated porous medium, which is not possible with pure clay because of its low hydraulic conductivity (*33*). In the present study, we employed a similar system to study the transport of different PAH-degrading bacteria through a clay-containing soil and the isolated soil constituents montmorillonite clay, sand, or soil humic acid-clay complexes. The bacterial strains tested belonged predominantly to the genera *Mycobacterium* and *Sphingomonas*, specialists in degrading the poorly available PAHs and potential inoculants for PAH bioremediation. Our main objectives were (i) to compare the effects of different soil constituents on the transport of these bacteria, (ii) to determine if the effects can be explained on the basis of specific properties of bacteria and/or soil components, and (iii) to find means for facilitating bacterial transport through soil.

Materials and Methods

Chemicals. Phenanthrene, anthracene, fluorene, fluoranthrene and pyrene were purchased from Sigma (Buchs, Switzerland). Polyvinyl alcohol (100 000) (PVA) was obtained from Fluka AG (Buchs, Switzerland). Glass beads (250−310 μm, mean diameter, 275 μm) were from Roth AG (Reinach, Switzerland).

Bacteria, Media, and Cultivation. The bacteria used in this study originated from PAH-contaminated soils and were able to grow with individual PAHs as the sole sources of carbon and energy (Table 1). The *Mycobacterium* strains LB307T and LB501T were isolated by a novel method using Teflon membranes containing sorbed PAHs for the enrichment and recovery of bacteria from soil (*19*). Two different media were used to grow the strains: a Tris minimal medium (*19*) for strains LH162, VM502, VM503 and VM505 and a phosphate-buffered medium (*34*) for *Arthrobacter polychromogenes* and strains LB126, LB208, LB307T and LB501T. The bacteria were routinely maintained in the mineral salts medium containing 0.2% of the respective PAH. For transport experiments, the bacteria were grown in 500 mL of mineral salts medium supplied with the PAH of choice at 30 °C on a rotary shaker at 180 rpm and harvested in the early stationary phase. Cultures were passed through a glass frit of 40-μm pore size to eliminate the remaining PAH crystals, and cells in the filtrate were harvested, washed twice in 10 mM phosphate-buffered saline (PBS) solutions composed of 0.493 g of NaCl, 0.029 g of KH_2PO_4, and 0.119 g of K_2HPO_4 per liter (pH 7.2), and resuspended in the same buffer. They were immediately used for transport experiments and for the determination of their physicochemical characteristics.

Characterization of Bacteria. Electrophoretic mobilities and water contact angles (θ_W) of bacterial cells were determined as described by van Loosdrecht et al. (*35*). A Doppler electrophoretic light-scattering analyzer (Zetamaster; Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom) was used to measure the electrophoretic mobility. Zeta potentials (ζ), that can be used as indirect estimate of cell surface charge, were calculated from the electrophoretic mobility according to the method of Helmholtz-Smoluchowski (*36*). To determine cell surface hydrophobicity, bacterial cells were collected on Micropore filters of 0.45-μm pore size (Schleicher & Schuell, Dassel, Germany). The filters were mounted on glass slides and dried for 2 h at room temperature. Cell surface hydrophobicity was quantified by measuring the contact angle between the cell layer and a drop of water, using a microscope equipped with a goniometric eyepiece (Krüss GmbH, Hamburg, Germany).

Materials. Soils used in this study were used either as intact soil matrices for transport experiments or as a source of selected soil components (humic fractions and sand). Soils and soil materials were characterized according to standard methods of soil analysis (*37, 38*). A loamy sand from Coria del Río, Seville, Spain (Typic Xerochrepts, 0.8% organic matter, pH 6.5) was used in transport experiments with whole soil. Its particle size distribution was 73.3% coarse-grained sand (2.0−0.2 mm), 6.0% fine-grained sand (0.20−0.05 mm), 14.0% silt (0.050−0.002 mm), and 6.7% clay (<0.002 mm). The soil was air-dried and sieved (2-mm mesh). To simulate conditions in polluted soils, portions of dry soil were artificially contaminated before packing with the PAH used to cultivate the test strain. The PAH was spiked as a methylene chloride solution, which was given time to evaporate, giving a final concentration of 2.8 mg/g dry soil. This relatively low concentration, which is typical for PAH-contamination of soils (*39, 40*), did not affect bacterial transport, as was revealed by control experiments without PAH.

Soil components were isolated from two sandy soils (Typic Humaquept) from Doñana National Park, Huelva, Spain: one from sites neighboring Santa Olalla lake (4.3% organic matter, pH 6.5) and the other from Pinar del Raposo (3.2% organic matter, pH 6.5). The sand fraction from the Santa Olalla soil was obtained by gravity settling in deionized water, which eliminated most of the organic matter originally present in the sample. Elemental analysis of this material yielded a carbon content of 0.17%, and its particle composition was 3.1% coarse-grained sand (2−0.2 mm) and 96.9% fine-grained sand (0.20−0.05 mm). Purified humic acid fractions were obtained after extraction with NaOH, precipitation with acid, and dialysis (*41*). ^{13}C NMR-analysis of these fractions gave results typical for soil humic substances, i.e., signals indicating carboxylic acid groups (200−170 ppm), aromatic groups

(160−100 ppm), and alkyl carbon (50−0 ppm). The results of elemental analysis (% w/w ash free) performed in a LECO CHNS932 elemental analyzer were, for Santa Olalla humic acid (HA1), C: 47.0%, H: 5.31%, O: 43.72%, N: 3.97%, and, for Pinar del Raposo humic acid (HA2), C: 54.84%, H: 5.52%, O: 35.77%, N: 3.87%. The lower C/O ratio of HA1 indicates that this was more polar than HA2 (*42*).

The humic fractions were sorbed onto montmorillonite clay (Swy-2, Univ Missouri-Columbia, MO) to obtain stable complexes. The clay had been purified beforehand by gravity settling in deionized water to remove particles larger than 2 μm, saturated with 1 M $CaCl_2$, dialyzed to remove excess anions, and lyophilized. For the preparation of complexes, 1.8 g of humic fraction were dissolved in 25 mL of 0.1 N NaOH by shaking the solutions in a rotary shaker for about 8 h. Then, 7 g of montmorillonite was added to the solution, and the mixture was kept in darkness overnight. The nonadsorbed humic fraction was washed out after repeated centrifugation and resuspension in distilled water until no color was visible in the supernatant. The remaining pellet was lyophilized. The fractional organic carbon content (f_{oc}), or mass fraction of organic carbon contributed by the sorbed humic fractions, was approximately 0.01, as determined by elemental analysis. Clay had undetectable levels of f_{oc}. For column experiments, the clay and humic acid-clay complexes were immobilized on glass beads by using PVA as aggregating agent. The resulting particles had diameters of 250 to 500 μm and contained 4% (w/w) immobilized clay (*33*, *43*). To have analogous conditions to those in whole-soil experiments, PAHs were spiked on the individual soil components as described above. Scanning electron micrographs of gold-sputtered portions of these materials, performed with a Philips XL 30 microscope (Philips, Eindhoven, The Netherlands), showed no significant changes in surface morphology due to PAH addition.

Column Experiments. Transport experiments were performed at 25 °C in percolated columns according to the method of Rijnaarts et al. (*44*). The test materials (soil, sand or clay aggregates immobilized on glass beads) were wet-packed in glass columns of 4.1 cm in length and 1 cm internal diameter. The amount of packing material present in each column was of the order of 3.5 g. The omission of nutrients in the suspensions prevented significant bacterial proliferation during the experiments. The columns were connected to a peristaltic pump, and suspensions of PAH-grown bacteria ($OD_{280} = 0.7$) were pumped through the columns at constant flow rates. Identical empty bed flow rates were used, leading to porosity-dependent different hydraulic flow rates. These were 0.947 cm/min for soil, which had a gravimetrically estimated porosity of 0.22, corresponding to a pore volume (PV) of 0.71 mL and a hydraulic residence time (t_R) of 4.33 min, 0.727 cm/min for sand (porosity, 0.26; PV, 0.84 mL; t_R, 5.64 min), 0.526 cm/min for clay aggregates (porosity, 0.37; PV, 1.20 mL; t_R, 7.79 min), 0.508 cm/min for HA1-clay aggregates (porosity, 0.41; PV, 1.32 mL; t_R, 8.07 min), and 0.540 cm/min for HA2-clay aggregates (porosity, 0.38; PV, 1.21 mL; t_R, 7.59 min). Column breakthrough of bacteria was followed photometrically at time intervals. The efficiency of bacterial removal was expressed as optical density (OD) at 280 nm in column effluents C divided by those in column influents C_0. Light microscopy confirmed the absence of clay particles in column effluents. Control experiments showed negligible sorption of all bacteria to glass beads coated with PVA only ($\alpha_t < 0.04$; for definition see below) and no effect of PAH on bacterial transport. All results are given as means of duplicate measurements. The maximum differences between individual columns were lower than 5%. Transport experiments with the nonionic surfactant Triton X-100 were performed with bacterial suspensions in PBS solution, containing the desired surfactant concentration, that were prepared immediately before the experiments. Neither the soil nor the soil materials were treated with the surfactant before pumping the bacterial suspensions through the columns.

Filtration was quantified for all combinations of bacteria and packing materials. The adhesion efficiency α_t is commonly defined as the ratio of the rate of attachment η_t to the rate of bacterial transport to the surfaces of the packing material η_{trans}

$$\alpha_t = \eta_t/\eta_{trans}$$

Its calculation, thus, relies on a knowledge of the transport rate. For columns packed with spheres of uniform size, a calculation method for η_{trans} taking into account the contributions of convection, diffusion, van der Waals attraction, and sedimentation has been proposed and its usefulness shown (*44*, *45*). To be able to compare the results obtained with individual bacteria and packing materials in our experiments, we used this equation to calculate adhesion efficiencies α_t although our packing materials were far from being ideal spheres. For the calculations, we assumed spheres of identical size (275-μm radius) in their closest packing, and effective bacterial radii $R = 0.5\ (wl)^{1/2}$, where w and l stand for the width and the length of the bacteria, respectively. η_t was calculated from C/C_0 values obtained in transport experiments according to Jucker et al. (*46*). For a detailed explanation of the calculation method used, the reader is referred to the work by Martin et al. (*47*) and the filtration equation of Rajagopalan and Tien (*48*). Due to increased contact probabilities with the irregular surfaces of the packing particles used in this study, values of α_t may exceed 1 and should be considered as relative adhesion efficiencies. In some experiments, mainly with soil and isolated clay, α_t values did not reach a constant value but dropped linearly, in general after 2 or 4 pore volumes. For this reason, and to account for the dynamics of filter blocking, two representative values for α_t were calculated: An initial one, calculated at the point of change of slope in C/C_0 vs PV plots corresponding to the end of the bacterial front, and a final one, corresponding to the end of the experimental period.

Results

Physicochemical Characteristics of Bacteria. The nine bacteria differed in surface zeta potential, hydrophobicity, and cell dimensions (Table 1). Although the cell surface properties of some of these strains have been examined in an earlier study (*19*), measurements were repeated within the framework of the present study to have the actual values from the cultures used in transport experiments. The differences between these two independent determinations were minor. According to a classification proposed elsewhere (*49*), the *Mycobacterium* strains LB208, LB307T, LB501T, VM502 and VM503, and *Sphingomonas* sp. LH162 are highly hydrophobic, with contact angle values above 70°, whereas *Sphingomonas* sp. LB126 and *Arthrobacter polychromogenes* are hydrophilic, with contact angle values below 35°. Under the experimental conditions, all strains have a negative zeta potential, ranging from the relatively low zeta potential of cells of *Sphingomonas* sp. LB126 to the extremely low zeta potential of *Mycobacterium* sp. LB501T cells. This anthracene-degrading bacterium combines a low zeta potential with a high hydrophobicity (*19*).

Transport of Bacteria through Saturated Soil. Cell suspensions pumped through columns containing loamy sand broke through (i.e., reached $0.5 \times C/C_0$ max) after one pore volume. Drastically different C/C_0 values, from 0.08 for strain VM503 to 0.72 for *Sphingomonas* sp. LH162, indicate a broad range of removal efficiency (Table 1). Relative adhesion efficiencies (α_t) were calculated using C/C_0 values.

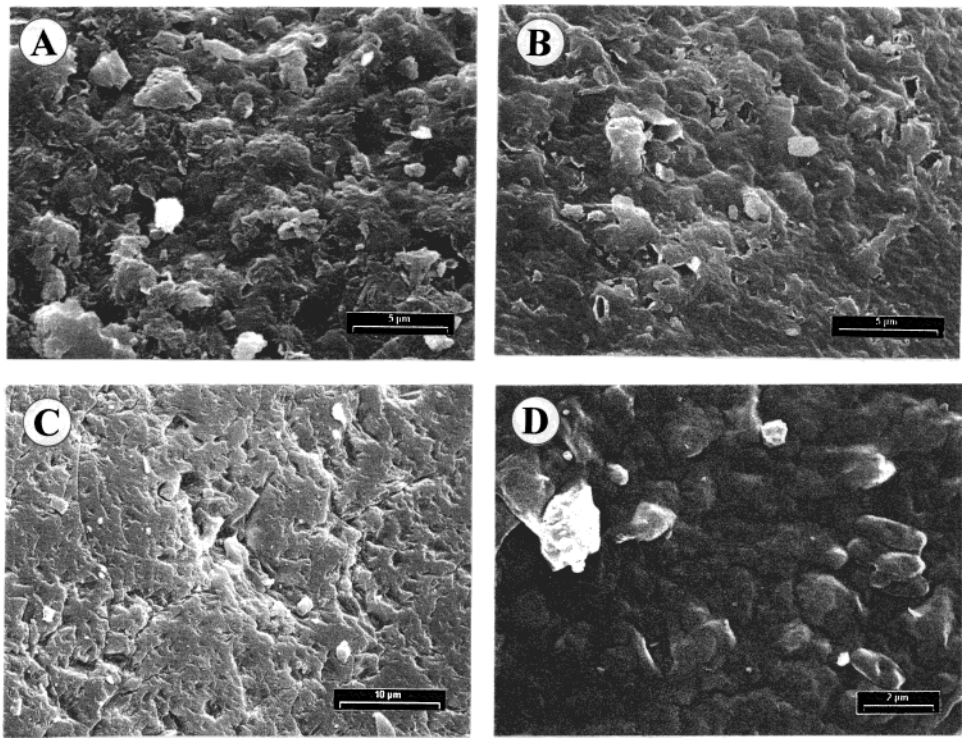

FIGURE 1. Scanning electron micrographs of clay aggregates (A), humic acid-clay aggregates (B), and sand (C) used in transport experiments with soil components. (D) biofilms formed after transport experiments by *Mycobacterium* sp. LB501T on aggregated clay.

Unlike C/C_0, which is an overall measurement for cell removal, the calculation of α_t takes into account differential frequencies of contact with the column material for bacteria having different sizes. Therefore, α_t represents the relative affinity of bacteria for the soil material. Values of α_t varied within a broad range (Table 1). However, no clear relationship was observed between any of the bacterial physicochemical characteristics examined and α_t. For example, the strains *Sphingomonas* sp. LH162 and *Mycobacterium* sp. VM503 exhibited a similar hydrophobicity but the lowest and the highest adhesion efficiency, respectively. The strains representative of different transport behavior in soil columns were selected for further experiments with isolated soil components.

Effect of Soil Constituents on Microbial Transport. Experiments involving clay materials were performed in columns containing glass beads coated with a mixture of PVA and clay. Scanning electron micrographs of these materials showed no differences in thickness of the layers formed on the glass surfaces by the aggregated clay and humic acid-clay complexes. They covered the glass surface discontinuously and had a thickness of up to 10 μm. However, the aggregated clay had a rougher surface than the aggregated humic acid-clay complexes (Figure 1).

Transport experiments with all combinations of bacteria and materials revealed that clay aggregates had the highest tendency to retain bacteria. Bacterial transport was, nevertheless, highly strain-specific (Table 2). With clay, α_t values were higher than with loamy sand for most strains tested, although the relative differences between strains observed in soil were basically conserved. This suggests that bacteria had a strong affinity to clay surfaces present in whole soil, despite its moderate clay content (6.7%), and even not being cation-saturated prior use. The cells of some strains were heavily accumulated within clay columns, leading to the formation of biofilms that completely covered the aggregated clay (Figure 1D). When clay surfaces were coated with either of the two different humic fractions, bacterial transport was greatly facilitated, as can be seen from the high C/C_0 values. Coating of clay with humic acid did not significantly change the bulk porosity of the material or the overall stereometry of the packings, which could have accompanied changed collision probabilities of bacteria with surfaces. It seems, therefore, that sorbed humic acid interferes with bacterial association to clay. Considerably lower values of α_t (Table 2) were obtained with sand than with clay aggregates, although both the porosity and the hydraulic conductivity of the sand were significantly lower than those of the packings of clay aggregates glued to glass beads. This confirms the assumption that clay is the main retaining agent of bacteria in whole soil.

Effect of Triton X-100 on Bacterial Transport. Two strains with high relative adhesion efficiency for loamy sand but different physicochemical cell surface characteristics, *Mycobacterium* sp. LB208 and *Sphingomonas* sp. LB126, were selected. Bacterial suspensions of these strains, containing different concentrations of Triton X-100, were pumped through columns containing loamy sand. The two strains responded differently to surfactant addition (Figure 2). Transport of the hydrophobic *Mycobacterium* sp. LB208 was clearly enhanced, with C/C_0 reaching unity at 500 μg/mL surfactant, whereas transport of the hydrophilic *Sphingomonas* sp. LB126 remained unchanged in the presence of the surfactant even at concentrations above its CMC (200 μg/mL).

The strain *Mycobacterium* sp. LB501T, having extreme cell-surface properties, exhibited the highest affinity toward clay and humic acid-clay surfaces (Table 2). Therefore, an experiment was conducted to assess the effect of surfactant addition on sorption of this strain to soil and individual soil constituents. Triton X-100 at a concentration of 300 μg/mL decreased bacterial retention in soil and clay and to a lesser extent in humic acid-clay complexes (Figure 3). The progressive increase in C/C_0 values with surfactant suspensions pumped through clay columns can be ascribed to progressive surfactant sorption to the collector surfaces (*46*). Alternately, bacteria release or detachment could have caused this progressive increase in C/C_0 values, a possibility that could not be ruled out based on our results.

TABLE 2. Influence of Model Soil Surfaces on Transport through Saturated Columns, as Compared with Glass and Teflon[a]

bacterium	θ_w[b] (deg)	ζ[c] (mV)	clay[d]			humic acid 1-clay[d]			humic acid 2- clay[d]			sand[d]			glass		Teflon	
			C/C_0	α_t[e]	PV[f]	C/C_0	α_t[e]	PV[f]	C/C_0	α_t[e]	PV[f]	C/C_0	α_t[e]	PV[f]	C/C_0	α_t[e]	C/C_0	α_t[e]
Sphingomonas sp. LH162	77.0 (59.1)	−23.6 (−36.2)	0.62 (0.95)	0.384 (0.040)	1.73 (13.4)	1	g	g	1	g	g	0.63 (0.99)	0.238 (0.003)	2.94 (18.96)	0.98	0.028	0.98	0.029
Mycobacterium sp. LB 307T	85.7 (102.9)	−22.3 (−28.7)	0.47 (0.61)	0.798 (0.523)	1.43 (13.1)	1	g	g	1	g	g	1	g	g	0.37	0.454	0.17	0.846
Arthrobacter polychromogenes	28.2	−22.6	0.26 (0.31)	1.156 (1.015)	1.43 (13.1)	0.82 (0.98)	0.197 (0.015)	1.29 (12.4)	1	g	g	1	g	g	ND[h]	ND[h]	ND[h]	ND[h]
Mycobacterium sp. LB208	83.0 (88.3)	−40.6 (−44.1)	0.19 (0.24)	1.993 (1.726)	1.43 (13.1)	0.70 (0.91)	0.510 (0.138)	1.29 (12.44)	1	g	g	0.66 (0.97)	0.307 (0.016)	2.64 (18.66)	0.58	0.559	0.32	0.773
Sphingomonas sp. LB126	23.4 (35.5)	−2.6 (−11.03)	0.03 (0.07)	3.944 (3.127)	1.43 (13.1)	0.62 (0.97)	0.701 (0.035)	1.29 (12.4)	0.70 (0.96)	0.442 (0.045)	1.45 (13.18)	0.77 (0.99)	0.196 (0.003)	2.64 (18.66)	0.99	0.024	0.63	0.467
Mycobacterium sp. LB501T	75.9 (88.5)	−60.2 (−65.5)	0.01 (0.11)	3.075 (2.395)	1.53 (13.2)	0.27 (0.47)	1.702 (0.997)	1.89 (13.04)	0.48 (0.61)	0.831 (0.573)	2.05 (13.79)	0.57 (0.86)	0.379 (0.102)	4.22 (18.46)	0.32	0.488	0.19	0.820

[a] θ_w and ζ data in parentheses, and data of transport through glass and Teflon are from Bastiaens et al. (*19*). [b] θ_w, Contact angle. [c] ζ, zeta potential. [d] Final experimental values are given in parentheses as an indication of the dynamics of filter blocking. [e] α_t, adhesion efficiency. [f] PV, pore volume. [g] α_t values could not be determined with the column length used. [h] ND, not determined.

FIGURE 2. Effect of Triton X-100 on bacterial transport through loamy sand. A, *Mycobacterium* sp. LB208; B, *Sphingomonas* sp. LB126. Numbers refer to surfactant concentration in μg/mL.

Discussion

Column experiments indicated that individual soil solid components exert different influences on the transport of PAH-degrading bacteria. Clay surfaces significantly retained most of the bacteria studied. Because clay was used in form of aggregates, which were glued to relatively large glass beads, this cell retention was not dominated by direct interception, as would be expected for pure clay, but to the tendency of most bacteria to associate to clay surfaces. This is supported by (i) the slight retention of one of the strains, *Sphingomonas* sp. LH162, in clay-filled columns, (ii) the relatively big differences in the affinity for clay between individual strains, and (iii) the negligible tendency to retain bacteria of humic acid-clay aggregates, which had similar dimensions to clay aggregates. It is nevertheless possible that the irregular surfaces of the soil and soil components used in this study may have caused additional bacterial retention in the columns, different from that attributable to physicochemical interactions. This limitation does not invalidate the use of relative adhesion efficiencies as indicators of bacterial transport in this study, because this situation was applicable to all the strains tested. The fact that α_t values exceeded 1 only in columns with soil and isolated clay (which theoretically would not be possible with ideal spheres) suggests that some of the clay aggregates present were acting as a filter via an additional mechanism. It may well be that clay caused higher bacterial removal due to the combined effects of more efficient attachment of bacteria to the column packing and a higher contact probability between bacteria and the column packing.

Humic substances notably stimulated bacterial transport of strains that were retained by clay surfaces. From the

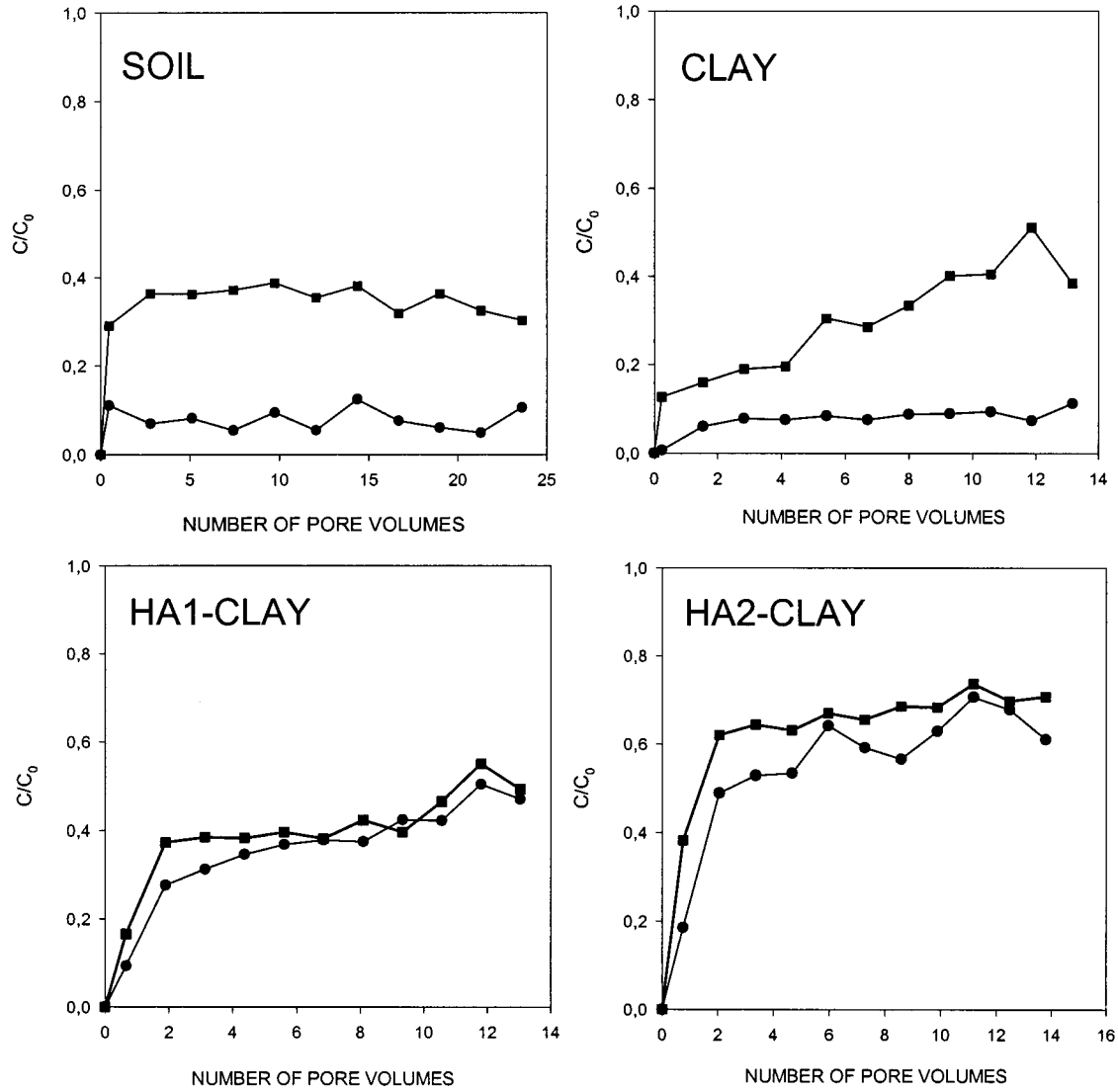

FIGURE 3. Effect of Triton X-100 at 300 μg/mL on transport of *Mycobacterium* sp. LB501T through saturated columns of soil, clay-coated glass, and humic acid-clay-coated glass. Squares and circles represent treatments with and without surfactant, respectively.

generally low retention of bacteria by humic acid-clay complexes (the strain *Mycobacterium* sp. LB501T, with extreme values for zeta potential and hydrophobicity, was an exception), it can be seen that association of most bacteria to humic acids is not favorable and that humic acids efficiently covered the underlying clay surfaces. These findings seem to be in disagreement with previous observations about the spatial distribution of bacteria in soil, which reported that 60% of the soil bacteria were attached to particles covered with organic matter, although these particles contributed only 15% to the total particle surface (*3*). The discrepancy may be resolved by assuming that most organisms bound to organic matter originate from growth in situ rather than from adhesion from the liquid phase. Upon multiplication, soil microorganisms most likely form microcolonies rather than releasing their descendants into the soil water. Organic matter may release the growth substrate of these attached bacterial populations and provide a favorable microclimate due to its water-holding capacity.

The bacterial strains used are negatively charged, as are the montmorillonite surfaces (due to isomorphous substitution in the octahedral sheet). At the ionic strength used in the experiments (10^{-2} M), this would lead to electrostatic repulsion (*32*). However, van der Waals attraction, shielding of coulomb forces by divalent cations and H-bridges between bacterial surface polymers and mineral surfaces may promote cell adhesion (*50*). In our study, these forces seemed to operate specifically with clay surfaces, because sand-filled columns were passed nearly unhindered by all bacteria. Only *Mycobacterium* sp. LB501T had a certain tendency to sorb to sand.

Although our experimental system is of limited similarity to soils, its results provide new insights into the interactions of bacteria and soil components. These results agree well with studies that showed a stimulatory effect of sediment-bound organic matter on bacterial transport (*29, 51*). They are, however, somewhat different to those obtained previously in similar column experiments with glass and Teflon (*19*), two materials that have been considered model representatives of hydrophilic (glass) and hydrophobic (Teflon) environmental surfaces (Table 2). Only the low affinity of the strain *Sphingomonas* sp. LH162 to these two materials agreed with the results obtained with soil components. The interactions of the other strains with glass and Teflon were not reproduced with soil materials. For example, the hydrophobic strain *Mycobacterium* sp. LB307T sorbed significantly to glass and Teflon but showed little or no affinity toward soil components, while *Sphingomonas* sp. LB126 had little affinity to glass and Teflon but was strongly retained in columns with soil and clay. This suggests that the adhesion mechanisms operating with these soil surfaces were different from those with Teflon and glass, where cell hydrophobicity

in general promoted adhesion. The nonionic surfactant Triton X-100 considerably facilitated the transport of *Mycobacterium* sp. LB208 through soil. In contrast, transport of *Sphingomonas* sp. LH162 through soil was almost unaffected by the presence of the surfactant. An influence on bacterial transport through soil can be explained by surfactant sorption to both the bacterial cells and the soil surface. On one hand, sorption of surfactant to the hydrophilic soil surfaces will likely increase their hydrophobicity because of the probable orientation of the hydrophobic moieties into the aqueous phase (*52*). This increased surface hydrophobicity may have introduced hydrophobic interactions (which seemed not to be operating in the absence of the surfactant) as a factor affecting bacterial transport through soil, in a similar way to what can be observed with Teflon. On the other hand, sorption of surfactant molecules to bacterial cells may occur with either the hydrophobic or the hydrophilic moiety, turning hydrophobic cells into hydrophilic ones and vice versa (*53*). According to this mechanism, the final result was therefore repulsion from soil surfaces and an increased transport of the hydrophobic cells of *Mycobacterium* sp. LB208, whereas transport of the hydrophilic strain *Sphingomonas* sp. LB126 was not improved, due to the remaining affinity of modified cell and soil surfaces. Our results agree with other studies where this surfactant has been described as being responsible for decreases in microbial association to solid (*54*) and liquid (*55*, *56*) surfaces.

Cell size, aspect ratio, hydrophobicity, and zeta potential were taken into consideration as properties that possibly affected bacterial transport through soil. With the exception of the anthracene-degrader *Mycobacterium* sp. LB501T, which had an extremely negative zeta potential, these traits were within the range of those previously observed in studies focused on bacterial adhesion (*35*, *57*). These earlier studies suggest that hydrophobicity is a key factor determining bacterial adhesion to solid surfaces. Transport of bacteria through soil can also be affected by their cell size (*15*) and shape (*14*). In our study, performed with clay-containing media, these conventionally used cell parameters could not be used to predict the transport of PAH-degrading strains. Our results indicate that the presence of clay surfaces may significantly impede microbial transport through saturated porous media and that this limitation can be overcome to a certain extent by the use of specific strains and/or by surfactant addition. The selection of appropriate strains and nontoxic surfactants therefore seems a reasonable step during the design of bio-augmentation strategies for decontamination of soils polluted with PAHs.

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Literature Cited

(1) Leahy, J. G.; Colwell, R. R. *Microbiol. Rev.* **1990**, *54*, 305−315.
(2) Costerton, J. W.; Lappin-Scott, H. M. *ASM News* **1989**, *55*, 650−654.
(3) Gray, T. R. G.; Parkinson, D. *The Ecology of Soil Bacteria*; Liverpool University Press: Liverpool, England, 1968.
(4) Stotzky, G. *Crit. Rev. Microbiol.* **1972**, *2*, 59−126.
(5) Wallace, H. R. In: *Plant Disease: an Advanced Treatise, Vol. II.*; Horsfall, J. G., Cowling, E. B., Eds.; Academic Press: New York, 1978; pp 181−202.
(6) Bosma, T. N. P.; Middeldorp, P. J. M.; Schraa, G.; Zehnder, A. J. B. *Environ. Sci. Technol.* **1997**, *31*, 248−252.
(7) Alexander, M. *Biodegradation and Bioremediation*; Academic Press: San Diego, CA, 1999.
(8) Madsen, E. L.; Alexander, M. *Soil. Sci. Soc. Am. J.* **1982**, *46*, 557−560.
(9) Gannon, J. T.; Tan, Y.; Baveye, P.; Alexander, M. *Appl. Environ. Microbiol.* **1991**, *57*, 2497−2501.
(10) Bitton, G.; Lahav, N.; Henis, Y. *Plant Soil* **1974**, *40*, 373−380.
(11) Breitenbeck, G.; Yang, H.; Dunigan, E. P. *Biol. Fertil. Soils* **1988**, *7*, 58−62.
(12) Smith, M. S.; Thomas, G. W.; White, R. E.; Ritonga, D. *J. Environ. Qual.* **1985**, *14*, 87−91.
(13) Scholl, M. A.; Harvey, R. W. *Environ. Sci. Technol.* **1992**, *26*, 1410−1417.
(14) Weiss, T. H.; Mills, A. L.; Hornberger, G. M.; Herman, J. S. *Environ. Sci. Technol.* **1995**, *29*, 1737−1740.
(15) Gannon, J. T.; Manilal, J. T.; Alexander, M. *Appl. Environ. Microbiol.* **1991**, *57*, 190−193.
(16) Devare, M.; Alexander, M. *Soil Sci. Soc. Am. J.* **1995**, *59*, 1316−1320.
(17) Aitken, M. D.; Stringfellow, W. T.; Nagel, R. D.; Kazunga, C.; Chen, S. H. *Can. J. Microbiol.* **1998**, *44*, 743−752.
(18) Dagher, F.; Déziel, E.; Lirette, P.; Paquette, G.; Bisaillon, J. G.; Villemur, R. *Can. J. Microbiol.* **1997**, *43*, 368−377.
(19) Bastiaens, L.; Springael, D.; Wattiau, P.; Harms, H.; deWatchter, R.; Verachtert, H.; Diels, L. *Appl. Environ. Microbiol.* **2000**, *66*, 1834−1843.
(20) Edmonds, R. L. *Appl Environ. Microbiol.* **1976**, *32*, 537−546.
(21) Madsen, E. L.; Alexander, M. *Soil Sci. Soc. Am. J.* **1982**, *46*, 557−560.
(22) Robson, A. D.; Loneragan J. F. *Aust. J. Agric. Res.* **1970**, *21*, 435−445.
(23) Chatel, D. L.; Greenwood, R. M.; Parker, C. A. In *International Congress of Soil Sciences, Trans. 9th., II;* 1968; pp 65−73.
(24) Stenström, T. A. *Appl. Environ. Microbiol.* **1989**, *55*, 142−147.
(25) Hekman, W. E.; Heijnen, C. E.; Burgers, S. L. G. E.; van Veen, J. A.; van Elsas, J. D. *FEMS Microbiol. Ecol.* **1995**, *16*, 143−158.
(26) Mills, A. L.; Herman, J. S.; Hornberger, G. M.; De Jesus, T. D. *Appl. Environ. Microbiol.* **1994**, *60*, 3300−3306.
(27) Fontes, D. E.; Mills, A. L.; Hornberger, G. M.; Herman, J. S. *Appl. Environ. Microbiol.* **1991**, *57*, 2473−2481.
(28) Jackson, A.; Roy, D.; Breitenbeck, G. *Water Res.* **1994**, *28*, 943−949.
(29) Johnson, W. P.; Logan, B. E. *Water Res.* **1996**, *30*, 923−931.
(30) Huysman, F.; Verstraete, W. *Soil Biol. Biochem.* **1993**, *25*, 83−90.
(31) Stotzky, G. In: *Interactions of Soil Minerals with Natural Organics and Microbes*; Huang, P. M., Schnitzer, M., Eds.; Soil Science Society of America: Madison, WI, 1986; pp 305−428.
(32) Theng, B. K. G.; Orchard, V. A. In *Environmental Impact of Soil Component Interactions, vol 2: Metals, Other Inorganics, and Microbial Activities*; Huang, P. M., Ed.; Lewis: Boca Raton, FL, 1995; pp 123−143.
(33) Ortega-Calvo, J. J.; Fesch, C.; Harms, H. *Environ. Sci. Technol.* **1999**, *33*, 3737−3742.
(34) Harms, H.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1994**, *60*, 2736−2745.
(35) van Loosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Schraa, G.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1987**, *53*, 1989−1901.
(36) Hiemenz, P. C. *Principles of Colloid and Surface Chemistry*; Marcel Dekker: New York, 1986.
(37) Klute, A. *Methods of Soil Analysis. Physical and Mineralogical Methods;* Soil Science Society of America: Madison, WI, 1986.
(38) Page, A. L.; Miller, R. H.; Keeney, D. R. *Methods of Soil Analysis. Chemical and Microbiological Properties*; Soil Science Society of America: Madison, WI, 1982.
(39) Wang, X.; Yu, X.; Bartha, R. *Environ. Sci. Technol.* **1990**, *24*, 1086−1089.
(40) Wilson, S. C.; Jones, K. C. *Environ. Pollut.* **1993**, *81*, 229−249.
(41) Schnitzer, M. In *Methods of Soil Analysis. Chemical and Microbiological Properties*; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; Soil Science Society of America: Madison, WI, 1982; pp 581−594.
(42) Lahlou, M.; Ortega-Calvo, J. J. *Environ. Toxicol. Chem.* **1999**, *18*, 2729−2735.
(43) Fesch, C. Ph.D. Dissertation, Swiss Federal Institute of Technology, Zürich, Switzerland, 1997.
(44) Rijnaarts, H. H. M.; Norde, W.; Bouwer, E. J.; Lyklema, J.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1993**, *59*, 3255−3265.
(45) Martin, R. E.; Bouwer, E. J.; Hanna, L. M. *Environ. Sci. Technol.* **1992**, *26*, 1053−1058.
(46) Jucker, B. A.; Harms, H.; Zehnder, A. J. B. *J. Bacteriol.* **1996**, *178*, 5472−5479.

(47) Martin, R. E.; Bouwer, E. J.; Hanna, L. M. *Environ. Sci. Technol.* **1992**, *26*, 1053−1058.
(48) Rajagopalan, R.; Tien, C. *AIChE J.* **1976**, *22*, 523−533.
(49) Bendinger, B.; Rijnaarts, H. H. M.; Altendorf, K.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1993**, *59*, 3973−3977.
(50) Jucker, B. A.; Zehnder, A. J. B.; Harms, H. *Environ. Sci. Technol.* **1998**, *32*, 2909−2915.
(51) Harvey, R. W.; George, L. H.; Smith, R. L.; LeBlanc, D. R. *Environ. Sci. Technol.* **1989**, *23*, 51−56.
(52) Edwards, D. A.; Adeel, Z.; Luthy, R. G. *Environ. Sci. Technol.* **1994**, *28*, 1550−1560.
(53) Neu, T. R. *Microbiol. Rev.* **1996**, *60*, 151−166.
(54) Paul, J. H.; Jeffrey, W. H. *Can. J. Microbiol.* **1985**, *31*, 224−228.
(55) Stelmack, P. L.; Gray, M. R.; Pickard, M. A. *Appl. Environ. Microbiol.* **1999**, *65*, 163−168.
(56) Ortega-Calvo, J. J.; Alexander, M. *Appl. Environ. Microbiol.* **1994**, *60*, 2643−2646.
(57) Wollum, A. G.; Cassel, D. K. *Soil Sci. Soc. Am. J.* **1978**, *42*, 72−76.

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